# Biologically active secondary metabolites from Asteraceae and Polygonaceae species

Summary of Ph.D. Thesis

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**INTRODUCTION**

Cancer and cardiovascular diseases are the leading causes of death in the western world. Cancer and certain cardiovascular diseases, such as atherosclerosis, are often associated with inflammation, and it has been demonstrated that chronic inflammation may be the common factor in many diseases. The clinically used anti-inflammatory drugs (steroids) are effective, but their long-term use may require increasing doses and cause unwanted side-effects, such as hypertension, oedema, ulcer, weight gain and insulin resistance. There are other types of drugs that are non-steroidal anti-inflammatory agents (NSAIDs) and have lower toxicity. The treatment of cardiovascular diseases includes a series of indications, such as cardiotonic and antiarrhythmic agents, lowering the level of low-density lipoprotein cholesterol, decreasing the blood pressure, preventing blood clots, relieving fluid buildup and managing stress hormones. Great efforts are ongoing worldwide in the search for new compounds that can selectively influence these diseases.

Plants have a long history of use in the treatment of the above diseases. Over 60% of the currently used anticancer agents are derived from natural sources. The agents include vinblastine, vincristine, the camptothecin derivatives, etoposide and paclitaxel. Trabectedin (Yondelis®), isolated from the sea squirt, *Ecteinascidia turbinata*, provided the first marine anticancer drug to be approved in Europe. Ingenol-3-angelate (Picato®), a metabolite found in *Euphorbia peplus*, has approved for the treatment of actinic keratosis. Several plant-derived agents are currently undergoing clinical development, among them flavopiridol, combretastatins and roscovitine.

The best-known example of a natural anti-inflammatory agent is salicylic acid, isolated from *Salix* species. Cannabidiol, a secondary metabolite in *Cannabis sativa* has also been approved for the treatment of inflammation in many countries in 2005. In contrast to NSAIDs that inhibit the enzyme cyclo-oxygenase, the mode of action underlying the anti-inflammatory effects of natural products, such as sesquiterpene lactones (SLs) which has been explained by mechanisms involving the inhibition of nuclear factor-kB (NF-κB) and the production of inflammatory cytokines. This has raised interest in SLs as prospective therapeutics for the treatment of inflammation.

A number of bioactive compounds generally obtained from terrestrial plants such as isoflavones, resveratrol, quercetin, catechin, sulforaphane, tocotrienols and carotenoids have been proven to promote cardioprotection and to reduce the risk of cardiovascular diseases. The cardioprotective effects of the various phytochemicals may be due to their antioxidative, antihypercholesterolaemic, antiangiogenic, anti-ischaemic, platelet aggregation-inhibitory and anti-inflammatory activities that reduce the risk of cardiovascular disorders.

Natural products possess a broad diversity of structures and functions, and have traditionally provided substantial inspiration for drug development programs. Modern isolation and screening technologies have enhanced the search for new lead molecules and increased interest in folk-medicinal plant extracts. Secondary metabolites of the Asteraceae and Polygonaceae species can be regarded as promising starting materials for pharmaceutical discoveries, in consequence of their pharmacological potential, and in particular their noteworthy antitumour and anti-inflammatory effects, which provides a rationale for screening for new active constituents from these families for the treatment of cancer and cardiovascular disorders.

**AIMS OF THE STUDY**

A few years ago, the research group of the Department of Pharmacognosy at the University of Szeged started a screening programme to investigate the antiproliferative activity of the species of the Asteraceae family and to identify the bioactive compounds in the selected plants. The aim of the present work as part of this project was the chemical investigation and detailed pharmacological analysis of two species belonging to the Asteraceae family. In the course of the work, pharmacological screening was extended to plants of the Polygonaceae family.

In order to achieve the aims, the main tasks were:

- A review of the literature on the Asteraceae and Polygonaceae families, from aspect of the chemistry and pharmacological properties of the plants.
- Extraction of plant materials of Polygonaceae species with various solvents for the screening, and examination of the tumour cell proliferation-inhibitory and GIRK channels modifying activities of the extracts.
- Identification of the bioactive secondary metabolites of *Neurolaena lobata*: isolation, structure elucidation and *in vitro* and *in vivo* evaluation of antiproliferative and anti-inflammatory potential of the extracts and isolated compounds.
- Phytochemical and pharmacological analysis of *Onopordum acanthium*: isolation, structure determination of the compounds and *in vitro* anti-inflammatory evaluation of the extracts and compounds (including compounds previously isolated from the roots of *O. acanthium*).
- Isolation and structure determination of biologically active compounds from *Polygonum persicaria,* and liquid chromatographic−mass spectroscopic (LC-MS) investigation of samples of various origins from different vegetation stages**.**

**MATERIALS AND METHODS**

For antiproliferative and GIRK channel inhibitory screening, Polygonaceae species were collected in the flowering period between June and September 2010, in different regions of the Carpathian Basin (Croatia, Hungary and Romania). For preparative phytochemical work, aerial parts of *N. lobata* were collected in the area of the Chakmamantokrock formation and in the botanical garden of the Institute for Ethnobiology, San José, Guatemala in February 2011. Aerial parts of *O. acanthium* were collected in Kiskundorozsma (Hungary) in May 2008 and aerial parts of *P. persicaria* were collected in the flowering period in Szarvas-Cserebökény (Hungary) in June 2010. For the LC-MS investigation, *P. persicaria* samples were collected in Bélbor, Romania (flowering plant, collected in July 2012), in Szarvas-Furugy, Hungary (before the flowering period, June 2012), and in Homoródalmás, Romania (before the flowering period, in July 2012).

The air-dried plant materials were ground and percolated with MeOH. The concentrated extracts were diluted with $H_2O$, and the solutions were extracted first with *n*-hexane or petroleum ether, then with $CHCl_3$ or $CH_2Cl_2$ and finally with EtOAc (in case of *N. lobata*) to furnish fractions of different polarity. For the screening, $H_2O$-soluble extracts were also prepared.

The compounds were isolated by multistep chromatographic methods, including open-column chromatography (OCC), vacuum-liquid chromatography (VLC), rotation planar chromatography (RPC), medium-pressure liquid chromatography (MPLC), preparative layer chromatography (PLC), gel filtration (GF) and high-performance liquid chromatography (HPLC). Normal- or reversed-phase $SiO_2$, $Al_2O_3$ or Sephadex LH-20 were applied as stationary phases.

The isolated compounds were characterized and their structures were elucidated by means of different spectroscopic methods (NMR, HRESIMS, APCIMS and EIMS). For the chromatographic separations of *P. persicaria* samples were used a Shimadzu LC system.

In the course of the screening studies, antiproliferative effects were measured on 3 human cell lines [HeLa (cervix adenocarcinoma), MCF-7 (breast adenocarcinoma) and A-431 (skin epidermoid carcinoma)] with the MTT assay. The extracts of Polygonaceae species and compounds of aerial parts of *P. persicaria* were examined for their possible G protein-activated inwardly rectifying $K^+$ (GIRK) channel-inhibitory activity on HEK293 (human embryonic kidney) cells.

The compounds of *N. lobata* were tested for antiproliferative activity *in vitro* against human tumor cell lines (A2780 (ovarian carcinoma), A431, HeLa and MCF7). The effects of the $CH_2Cl_2$ extract and the compounds on the generation of pro-inflammatory proteins (IL-8 and E-selectin) after stimulation with LPS and TNF-α were assessed *in vitro* in endothelial (HUVECtert) and

monocytic (THP-1) cells. The activity of compounds on the relative mRNA expression of the IL-8 and E-selectin stimulated with LPS in HUVECtert cells were also analysed. Further, the anti-inflammatory action of extract was investigated *in vivo* by means of a rat paw oedema test.

The *in vitro* anti-inflammatory activity of extracts and compounds (including previously isolated compounds from the roots) of *O. acanthium* were evaluated by COX-2 and NF-κB1 gene expression, iNOS, 5-LOX, and COX-1 and COX-2 enzymes inhibitory assays. Cytotoxicity of the compounds were investigated by the XTT assay.

## RESULTS AND DISCUSSION

### SCREENING OF POLYGONACEAE SPECIES FOR ANTIPROLIFERATIVE AND GIRK CHANNEL INHIBITORY ACTIVITIES

In our screening, the antiproliferative effects of 27 species belonging in the *Fallopia* (3), *Oxyria* (1), *Persicaria* (2), *Polygonum* (8) and *Rumex* (13) genera of the Polygonaceae family were evaluated *in vitro* against three human tumour cell lines (HeLa, A431 and MCF7), using the MTT assay. The extracts prepared with *n*-hexane (A), $CHCl_3$ (B), aqueous MeOH (C) or $H_2O$ (D) from selected plant organs (altogether 196 extracts) were tested at concentrations of 10 μg/mL and 30 μg/mL.

In the course of this study, 6 species of the 27 tested plants exerted ≥50% inhibition of proliferation. For 16 species, a moderate (25–49.99%) cell growth inhibition was detected, while 5 plants were found to have no antiproliferative effects. Extracts of *Polygonum hydropiper, Rumex acetosa*, *R. alpinus*, *R. aquaticus*, *R. scutatus* and *R. thyrsiflorus* demonstrated substantial cell growth inhibitory activity (≥50%) at 10 or 30 μg/mL against one or more cell lines. These active extracts mostly originated from the roots of the plants, and among them fractions A and B proved to be active. *R. acetosa* (77.67% and 97.02% at 10 and 30 μg/mL on HeLa cells) and *R. thyrsiflorus* (96.20% and 88.55% at 30 μg/mL on A431 and MCF7 cells) were the most potent species; their phytochemical and pharmacological investigations are in progress.

For *P. hydropiper*, *R. acetosa*, *R. alpinus* and *R. aquaticus* the measured antiproliferative activities are in accordance with the traditional use of the plants against cancers. Other species, such as *Oxyria digyna*, *Persicaria amphibia*, *P. maculosa*, *Polygonum aviculare*, *P. bistorta*, *Rumex crispus*, *R. hydrolapathum* and *R. obtusifolius* exerted only moderate activities on the cell lines used, in spite of their traditional use in cancer treatment. On the other hand, the extracts of *R. scutatus* and *R. thyrsiflorus* demonstrated a strong anticancer profile, although their ethnomedicinal use has not been described previously.

In the course of GIRK channel inhibitory activity investigation, 51 extracts [*n*-hexane (A), $CHCl_3$ (B) and aqueous MeOH (C)] of 11 species were tested at 0.01 and 0.1 mg/mL concentrations on

human embryonic kidney. Among them mainly the $CHCl_3$ (B) extracts proved to be the most active ones; *P. aviculare* (75 ± 5%), *P. amphibia* (70 ± 12%), *P. persicaria* (76 ± 8%), *R. stenophyllus* (72 ± 3%), *R. patientia* (74 ± 2%) and *R. crispus* (72 ± 2°%) showed higher than 70% inhibitory activity at 0.1 mg/mL on GIRK channels (unpublished data). The best of our knowledge, this was the first application of the GIRK channel-inhibitory assay for the screening of plant extracts.

#### INVESTIGATION OF *N. LOBATA*, *O. ACANTHIUM* AND *P. PERSICARIA* EXTRACTS FOR BIOACTIVITY

Extracts of different polarity (aqueous and organic) prepared from the aerial parts of *N. lobata* have been tested before our work by KRUPITZA et al. (our cooperative partner) in human promyelocytic leukaemia cells (HL-60) with analyses of the inhibition of cell proliferation and apoptosis induction. The most active extract was further tested against anaplastic large cell lymphoma (ALCL) cell lines of human and mouse origin. The $CH_2Cl_2$ extract inhibited the proliferation of HL-60, human and mouse ALCL cells with an $IC_{50}$ of ~2.5, 3.7 and 2.4 µg/mL, respectively, and arrested cells in the G2/M phase.

The extracts of *O. acanthium* were evaluated for their inhibitory activity on COX-2 and NF-κB1 gene expression, iNOS, 5-LOX, and, COX-1 and COX-2 enzymes at 10 or 50 µg/mL in *in vitro* assays. In most cases, the $CHCl_3$ extract exerted strong inhibitory effects [inhibition of iNOS (76.67 ± 6.98%), 5-LOX (62.57 ± 6.84%), and COX-2 enzymes (61.75 ± 8.98%)]. Additionally, the effects of different *P. persicaria* extracts on the GIRK channel were investigated by using an automated patch clamp method. The $CHCl_3$ extract at 0.1 mg/mL exhibited significant GIRK channel-inhibitory activity (76 ± 8%).

On the basis of the results of the preliminary screening, the lipophilic extracts ($CHCl_3$ and $CH_2Cl_2$) were chosen for more detailed phytochemical studies, with the aim of the identification of their bioactive constituents.

#### ISOLATION OF BIOLOGICAL ACTIVE COMPOUNDS FROM *N. LOBATA*, *O. ACANTHIUM* AND *P. PESRSICARIA*

In the initial step of the phytochemical work, the dried plant materials were percolated with MeOH at room temperature; solvent–solvent extraction was then applied, which resulted in the $CHCl_3$ or $CH_2Cl_2$ phases. All of them were subjected to a multistep chromatographic procedure in order to isolate the compounds.

**Isolation of compounds from *Neurolaena lobata***

The purification of the $CH_2Cl_2$-soluble phase of *N. lobata* was performed with open column chromatography (OCC) on polyamid, affording 7 main fractions (BI-BVII); among them, fraction BII was the most interesting (**Figure 1**). Since this fraction demonstrated a chemical complexity, more selective methods (VLC, RPC and PLC) were applied, with the use of silica gel and different solvent systems. Finally, NP- and RP-PLC separations were used for the isolation of compounds [**LOB-2**, **3**, **5**, **6**, **9–11**, **13–15**, **18**, **20** and **26**, (**1–13**)].

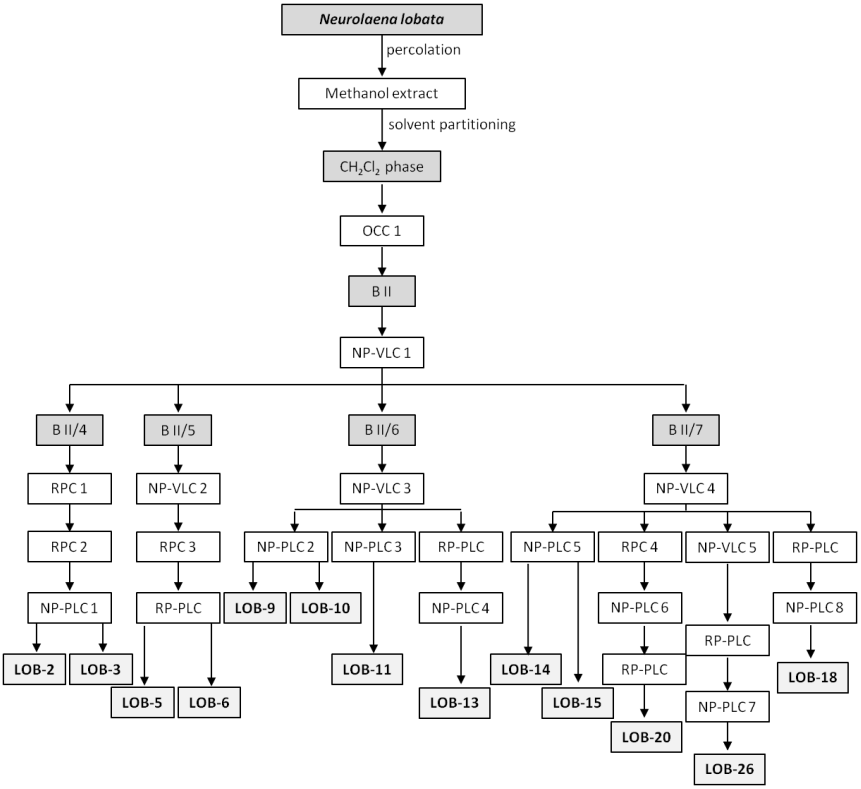



**Figure 1.** Isolation of compounds from *N. lobata*

**Isolation of compounds from *Onopordum acanthium***

The $CHCl_3$ phase of *O. acanthium* was separated by VLC to furnish 6 main fractions (BI-VI) (**Figure 2**). These fractions were evaluated for their inhibitory effects on COX-2 and NF-κB1 gene expression, iNOS, 5-LOX, and COX-1 and COX-2 enzymes in *in vitro* assays. Fractions BI, BIV and BV at 10 or 50 µg/mL exhibited significant or moderate activity in the inhibition of COX-2 gene expression(45.5 ± 8.3%, 31.5 ± 11.1% and 12.6 ± 5.7%), NO production (62.5 ± 16.5%, 102.0 ± 0.3% and 79.9 ± 6.2%) and COX-2 enzyme (63.8 ± 9.8%, 19.9 ± 8.4% and 44.9 ± 8.8%). Fraction BI was separated by CC on polyamide to give 7 subfractions (BI/1-I/7). The most active subfractions BI/2, BI/6 and BI/7 were then subjected to multiple chromatographic separations, including VLC, RPC, MPLC, gel filtration on Sephadex LH-20 and PLC. This purification process led to the isolation of 7 compounds [**OPD-2**–**5**, **6/A**, **6/B** and **8**, (**14**–**20**)].

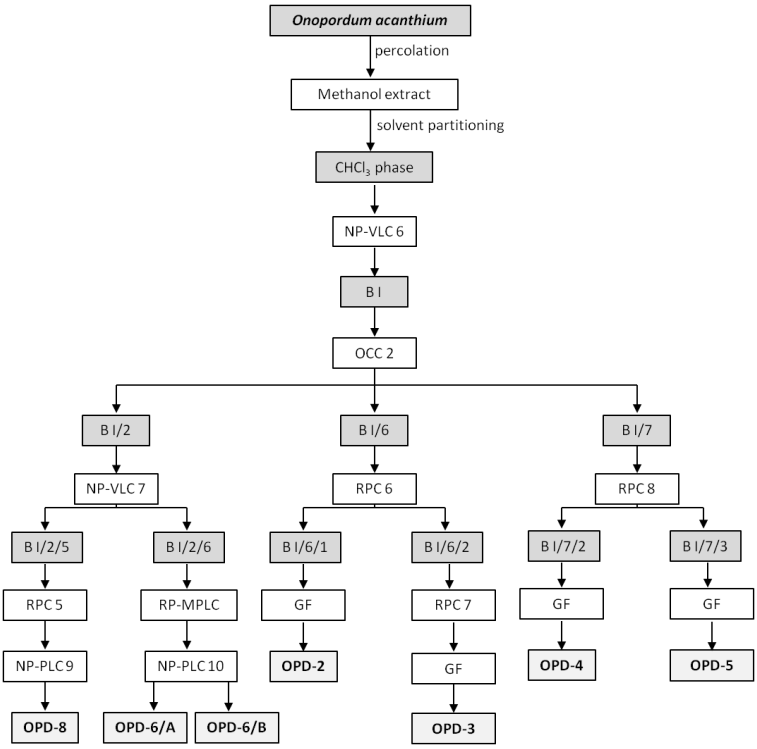



**Figure 2**. Isolation of compounds from *O. acanthium*

**Isolation of compounds from *Polygonum persicaria***

In the case of *P. persicaria*, the $CHCl_3$ phase was fractionated by RP-VLC on silica gel (**Figure 3**). The fractions were combined into 6 main fractions (B/1-B/6), and were tested for GIRK channel-inhibitory activity. Fractions B/4 and B/5 displayed considerable activity, which were further separated by RP-HPLC, to yield 4 compounds (**PP-1**–**4**, **21**–**24**) and a mixture containing the minor constituents.

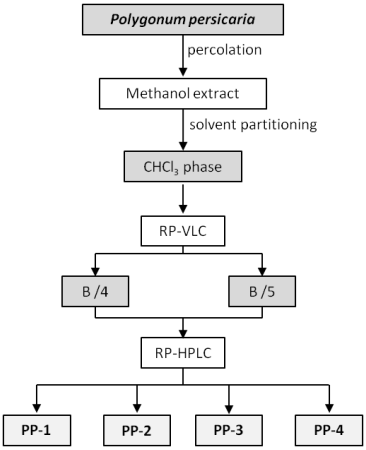


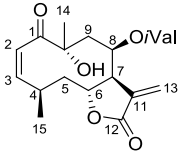

**Figure 3.** Isolation of compounds from *P. persicaria*

**CHARACTERIZATION AND STRUCTURE DETERMINATION OF ISOLATED COMPOUNDS**

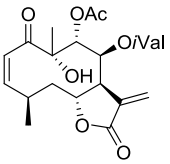
The chemical structures of the isolated compounds were determined by means of spectroscopic methods. The molecular masses and compositions were obtained from MS investigations; UV spectroscopic and optical rotation measurements provided further important information for characterization of the compounds.The most useful data concerning the structures were furnished by 1D and 2D NMR spectroscopy. The constitutions of the compounds were elucidated via $^{1}$H-NMR, JMOD, $^{1}$H-$^{1}$H COSY, HSQC and HMBC experiments, and the relative configurations were then characterized with the aid of NOESY spectra. As a result of the NMR studies, complete $^{1}$H- and $^{13}$C-assignments were made for the new compounds and also in the case of some known compounds, where previously published data were incomplete.

**Compounds from *Neurolaena lobata***

Thirteen SLs esterified with isovaleric acid at C-8 or C-9 (in case of **LOB-6**) were isolated from *N. lobata*. Eight of them, the germacranolide type **LOB-2** (neurolenin A, **1**), **LOB-3** (neurolenin B, **2**), **LOB-5** (neurolenin D, **3**), **LOB-6** (neurolenin C, **4**) and **LOB-10** (lobatin A, **6**) and the furanoheliangolide-type **LOB-11** (lobatin B, **7**), **LOB-9** (8β-isovaleryloxy-9α-acetoxy-calyculatolide, **5**) and **LOB-13** (8β-isovaleryloxy-9α-hydroxy-calyculatolide, **8**), had already been isolated from this species. **LOB-15** (**9**) and **LOB-14** (**10**) are unusual isomeric seco-germacranolide sesquiterpenes with a bicyclic acetal moiety. **LOB-18** (**11**), is an 1-keto-furanoheliangolide derivative similarly to **LOB-13** (**8**), from which it differs only at the substitution of C-5.




**1**

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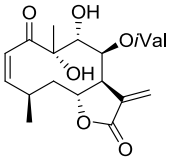


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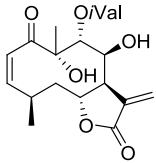


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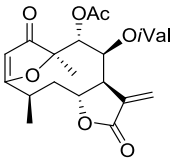


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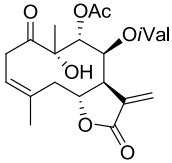


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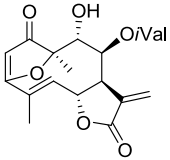


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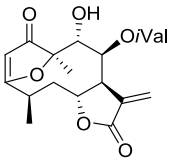


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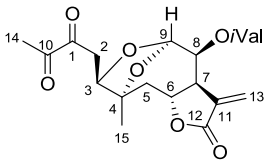


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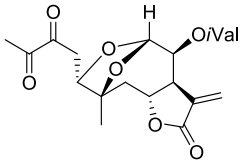


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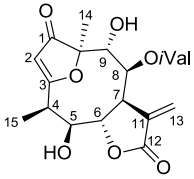


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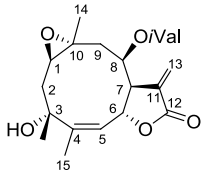


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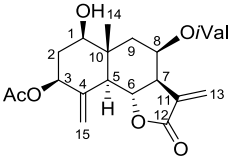
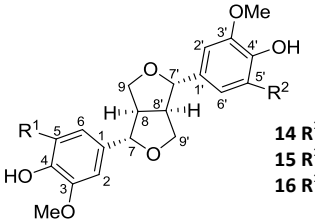



**13**

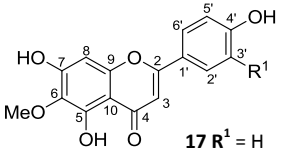
**LOB-20** (**12**), also an unsaturated epoxy-germacranolide ester, is the 3-epimer of desacetylisovaleroylheliangine, with differences in the $^{1}$H-NMR chemical shifts and the coupling constants of H-3. Structurally, **LOB-26** (**13**) is an eudesmanolide-type SL. Eudesmanolides occur widely in the family Asteraceae, but **LOB-26** (**13**) is the first isolated from the genus *Neurolaena*. **LOB-15** (neurolobatin A, **9**), **LOB-14** (neurolobatin B, **10**), **LOB-18** (5β-hydroxy-8β-isovaleroyloxy-9α-hydroxycalyculatolide, **11**), **LOB-20** (3-*epi*-desacetylisovaleroylheliangine, **12**) and **LOB-26** (3β-acetoxy-8β-isovaleroyloxyreynosin, **13**) were identified as new SLs.

**Compounds from *Onopordum acanthium***

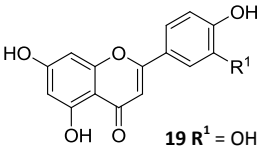
The structure analysis of compounds isolated from *O. acanthium* led to the identification of 3 lignans and 4 flavonoids. All of them [**OPD-8** ((+)-pinoresinol, **14**), **OPD-6/A** ((±)- syringaresinol, **15**), **OPD-6/B** (medioresinol, **16**) , **OPD-2** (hispidulin, **17**), **OPD-3** (nepetin, **18**), **OPD-4** (luteolin, **19**) and **OPD-5** (apigenin, **20**)] were identified on the basis of the comparison of the measured and literature MS and NMR data. In the case of **OPD-8** (**14**), In the case of **OPD-8** (**14**), the previously published NMR chemical shifts recorded in acetone were supplemented with complete $^{1}$H- and $^{13}$C-NMR assignments in $CDCl_3$. The compounds, excluding luteolin (**19**) and apigenin (**20**), were isolated for the first time from this species; furthermore, medioresinol (**16**) was also detected for the first time in the genus.




**14** **R**$^{1}$ = H; **R**$^{2}$= H
**15** **R**$^{1}$ = OMe; **R**$^{2}$ = OMe
**16** **R**$^{1}$ = OMe; **R**$^{2}$ = H




**17** **R**$^{1}$ = H
**18** **R**$^{1}$ = OH

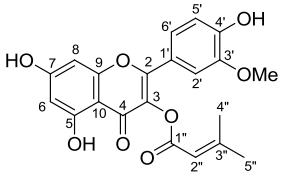



**19** **R**$^{1}$ = OH
**20** **R**$^{1}$ = H

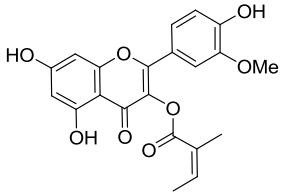
**Compounds from *Polygonum persicaria***

Four flavonoids were isolated from *P. persicaria*, among them, **PP-1** (**21**) and **PP-2** (**22**) being esterified at C-3. In the case of **PP-1**, the unusual senecioyl group is present in the molecule, while **PP-2** contains an angeloyl group. The presence of compounds containing a senecioyl group is very rare in the plant kingdom. **PP-3** (**23**) and **PP-4** (**24**) were identified as 6,7-methylenedioxyflavones and their characteristics are the presence of 4 or 5 methoxy groups in the molecule. **PP-1** (3-*O*-senecioyl-isorhamnetin, **21**), **PP-2** (3-*O*-angeloyl-isorhamnetin, **22**), **PP-3** (5,3',4',5'-tetramethoxy-6,7-methylenedioxyflavone, **23**) and **PP-4** (3,5,3',4',5'-pentamethoxy-6,7-methylenedioxyflavone, **24**) are new natural products, though **PP-4** was reported earlier as a synthetic compound.

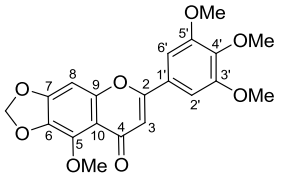
LC-MS investigation of the occurrence of compounds **21**–**24** in *P. persicaria* samples of various origins in different vegetation stages revealed that only samples collected in the flowering period contained these flavonoids.



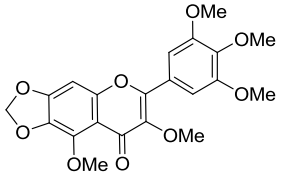
**21**



**22**



**23**



**24**

**BIOLOGICAL ACTIVITIES**

***Neurolaena lobata***

The new SLs (**9**–**13**) from the active extract ($CH_2Cl_2$) of *N. lobata* were tested for antiproliferative activities against human tumor cell lines (A2780, A431, HeLa and MCF7). All these compounds except **11** inhibited the proliferation of A431 and A2780 cells, and were less active against MCF7 and HeLa cells. The $IC_{50}$ values of **10**, **12** and **13** against A431 (6.8 ± 0.56 μM, 7.2 ± 0.99 μM and 5.3 ± 0.47 μM) and MCF7 (7.2 ± 0.57 μM, **12**) cells were comparable to those of the

reference agent cisplatin (8.8 ± 0.97 μM on A431, and 8.0 ± 1.1 μM on MCF7). The anti-inflammatory effects of **9**–**13** were evaluated *in vitro* using in LPS- and TNF-α-induced IL-8 expression inhibitory assays in endothelial cells, and it was found that all these compounds strongly down-regulated the LPS-induced production of IL-8 protein, with neurolobatin B (**LOB-14**, **10**) and 3-*epi*-desacetylisovaleroylheliangine (**LOB-20**, **12**) being the most effective. Moreover, in contrast to the positive control BAY, the isolated compounds were selective as they inhibited only the LPS-induced IL-8 production.

The effects of the $CH_2Cl_2$ extract and the known SLs (**1**–**8**) on the generation of pro-inflammatory proteins were also assessed *in vitro* in endothelial and monocytic cells. At the highest tested concentration (10 μM), all of the compounds (**1–8**) strongly decreased the secretion of IL-8 in LPS-stimulated endothelial cells. The most active compounds, neurolenin B (**LOB-3**, **2**), lobatin B (**LOB-11**, **7**) and 8β-isovaleryloxy-9α-acetoxy-calyculatolide (**LOB-9**, **5**) also down-regulated the production of IL-8 protein in TNF-α-induced endothelial cells. The extract (5 μg/mL) and the 8 known compounds (5 μM) demonstrated significant effects on another inflammation marker, the adhesion molecule E-selectin after stimulation with LPS and TNF-α. In order to test whether *N. lobata* components modulate the expression of inflammatory genes at the mRNA level, endothelial cells were treated with the three most active SLs, **LOB-3** (**2**), **LOB-9** (**5**) and **LOB-11** (**7**) The most active SLs were also tested on the relative mRNA expression of the IL-8 and E-selectin genes after stimulation with LPS in the endothelial cells. The relative mRNA expression of the IL-8 and E-selectin genes in the endothelial cells was strongly inhibited by the SLs as compared with activation with LPS alone.

Lobatin B (**LOB-11**, **7**) showed the most potent anti-inflammatory effect, comparable to those of the known inhibitors BAY and parthenolide. This was followed by 8β-isovaleryloxy-9α-acetoxy-calyculatolide (**LOB-9**, **5**), neurolenin B (**LOB-3**, **2**) and lobatin A (**LOB-10**, **6**). Moreover, the structure–activity analysis revealed the importance of the double bond at C-4–C-5 and C-2–C-3 and the acetyl group at C-9 for the anti-inflammatory activity.

The *in vivo* anti-inflammatory activity of the $CH_2Cl_2$ extract was evaluated by using a carrageenan-induced paw oedema model in rats. Both applied doses of *N. lobata* extract (20 and 60 mg/kg) inhibited the development of acute inflammation in rats. The suppression of local oedema formation by the higher dose was more that 50%. *In vivo* confirmation of the pharmacological effect raises further interest in the therapeutic potential of lobatin B (**LOB-11**, **7**) and related compounds.

***Onopordum acanthium***

The anti-inflammatory activities of the isolated compounds (**14**–**20**) from active $CHCl_3$ extract of aerial parts of the plant, together with the substances of the root extract [4β,15-dihydro-3-dehydrozaluzanin C (**25**), zaluzanin C (**26**), 4β,15,11β,13-tetrahydrozaluzanin C (**27**), nitidanin-diisovalerianate (**28**), 24-methylenecholesterol (**29**) and 13-oxo-9*Z*,11*E*-octadecadienoic acid (**30**)**]**, were tested at 20 μM on COX-2 and NF-κB1 gene expression, iNOS, 5-LOX, and COX-1 and COX-2 enzymes in *in vitro* assays. Among the flavonoids, noteworthy inhibitory activities (>50% inhibition) were recorded for hispidulin (**OPD-2, 17**), nepetin (**OPD-3**, **18**) and luteolin (**OPD-4**, **19**). Luteolin was the most potent in inhibition of 5-LOX (74.6 ± 8.8 %). As concerns the lignans, only moderate activities were observed for **14–16**. Two SLs, 4β,15-dihydro-3-dehydrozaluzanin C (**25**) and zaluzanin C (**26**), exhibited strong effects in COX-2 (98.6 ± 0.2% and 97.0 ± 1.1%) and NF-κB1 gene expression (78.7± 7.3% and 69.9 ± 3.4%), and NO assays (100.4 ± 0.5% and 99.4 ± 0.8%) at 20 μM. As far as we know, this is the first report on inhibitory activity of compounds **25** and **26** against COX-2 and NF-κB1 gene expression (mRNA level) in THP-1 cells. In order to determine, whether the gene expression-inhibitory effects were due to cytotoxicity, the compounds were investigated by the XTT assay at different time points (4, 24, 48 and 72 h) and at different concentrations. It was found, that the active compounds have no or low effects on cell viability at the tested concentrations.

***Polygonum persicaria***

The $CHCl_3$ extract of *P. persicaria* exhibited significant GIRK channel-inhibitory activity, its effect proving comparable to that of propafenone. The most effective fractions of the extract were B/4 and B/5, from which flavonoids (**21**–**24**) were isolated. Surprisingly, neither the individual, nor the combined application of the isolated compounds of the active fractions (**21**–**24**) exerted activity on the GIRK channel. However, the remaining HPLC eluates of fractions B/4 and B/5, containing mixtures of minor compounds, proved to have inhibitory activities of 63 ± 9% and 62 ± 4% at 0.1 mg/mL. The attempted isolation and identification of the compounds present in fractions B/4 and B/5 have so far failed because of their low quantities.

Our results reveal that secondary metabolites of Asteraceae and Polygonaceae species can be regarded as promising starting materials in the search for new pharmaceutical discoveries, in consequence of their pharmacological potential, and in particular their noteworthy anti-inflammatory and antitumour effects.

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**THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS:**

1. **Lajter I**, Zupkó I, Molnár J, Jakab G, Balogh L, Vasas A, Hohmann J.
   Antiproliferative activity of Polygonaceae species from the Carpathian Basin against human cancer cell lines
   *Phytother. Res.* 2013; **27**: 77-85. If: 2.397

2. **Lajter I**, Vasas A, Orvos P, Rédei D, Bánsághi S, Tálosi L, Jakab G, Béni Z, Háda V, Forgo P, Hohmann J.
   Inhibition of G protein-activated inwardly rectifying $K^+$ channels by extracts of *Polygonum persicaria* and isolation of new flavonoids from the chloroform extract of the herb
   *Planta Medica* 2013; **79**: 1736-1741. If: 2.339

3. **Lajter I**, Vasas A, Béni Z, Forgo P, Binder M, Bochkov V, Zupkó I, Krupitza G, Frisch R, Kopp B, Hohmann J.
   Sesquiterpenes from *Neurolaena lobata* and their antiproliferative and anti-inflammatory activities
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4. McKinnon R, Binder M, Zupkó I, Afonyushkin T, **Lajter I**, Vasas A, de Martin R, Unger C, Dolznig H, Diaz R, Frisch R, Passreiter CM, Krupitza G, Hohmann J, Kopp B, Bochkov VN. Pharmacological insight into the anti-inflammatory activity of sesquiterpene lactones from *Neurolaena lobata* (L.) R.Br. ex Cass.
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5. **Lajter I**, Pan SP, Nikles S, Ortmann S, Vasas A, Csupor-Löffler B, Forgó P, Hohmann J, Bauer R. Inhibition of COX-2 and NF-κB1 gene expression, NO production, leukotriene biosynthesis, and, COX-1 and COX-2 enzymes by extracts and constituents of *Onopordum acanthium*
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2. Orbán-Gyapai O, **Lajter I**, Hohmann J, Jakab G, Vasas A.
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*The impact factor for the year 2014 is given.

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1. **Lajter I**, Zupkó I, Molnár J, Jakab G, Balogh L, Hohmann J:
   Kárpát-medencében honos Polygonaceae fajok antiproliferatív hatásának vizsgálata tumor sejteken *in vitro*
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   A Polygonaceae növénycsalád kárpát-medencei fajainak antiproliferativ szűrővizsgálata tumorsejteken
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9. Pan SP, **Lajter I**, Vasas A, Bauer R, Hohmann J.
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